FORM PTO-1390 (REV 10-94) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER 7841-89

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/402446

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA98/00325	7 April 1998	7 April 1997

TITLE OF INVENTION

INTRAVENOUS IMMUNE GLOBULIN FORMULATION CONTAINING A NON-IONIC SURFACE ACTIVE AGENT WITH IMPROVED PHARMACOKINETIC PROPERTIES

APPLICANT(S) FOR DO/EO/US

Hugh W. Price and B. Michael R. Woloski

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   (a) [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   (b) [X] has been transmitted by the International Bureau.
   (c) [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   (a) [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   (b) [ ] have been transmitted by the International Bureau.
   (c) [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   (d) [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unsigned).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A SUBSTITUTE SPECIFICATION.

15. [ ] A CHANGE OF POWER OF ATTORNEY AND/OR ADDRESS LETTER.

16. [ ] Other items or information:

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/402446	PCT/CA98/00325	7841-89

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CF4 1.482) nor international search fee (37 CFR 1.445(a)(2)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00	
International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report prepared by EPO or JPO	$840.00	
International preliminary examination fee (37CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$760.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00	
International preliminary examination fee (37 CFR 1.482) paid to to USPTO and all claims satisfied provisions of PCT Article 33(2)-(4)	$96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$840.00

Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	- 20 =		x $18.00	$	
Independent Claims	- 3 =		x $78.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable) No			+ $260.00	$0	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$840.00	
				Amount to be refunded	$
				charged	$

a. [X] a check in the amount of $ $840.00 to cover the above fees is enclosed. (Cheque No. _____)

b. [ ] Please charge my Deposit Account No. 02-2095 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2095. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

M. Gravelle
Signature

SEND ALL CORRESPONDENCE TO:
Micheline Gravelle
Bereskin & Parr
Box 401, 40 King Street West
Toronto, Ontario, Canada M5H 3Y2

Micheline Gravelle
Name

40,261
Registration No.

Our Reference No. 7841-89

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Hugh W. Price and	)	Art Group No.: **Unknown**
B. Michael R. Woloski	)	
	)	
Serial No. **Unknown**	)	Examiner: **Unknown**
(U.S. National Phase of	)	
PCT/CA98/00325)	)	
	)	
Filed: **Concurrently herewith**	)	
	)	
For: **Intravenous Immune Globulin**	)	
Formulation containing a Non-	)	
Ionic Surface Active Agent With	)	
Improved Pharmacokinetic	)	
Properties	)	

The Commissioner of Patents & Trademarks
Washington, D.C. 20231
U.S.A.

Dear Sir:

PRELIMINARY AMENDMENT

We are simultaneously entering National Phase of PCT/CA98/00325 filed on April 7, 1998, claiming priority from United States Provisional Patent Application No. 60/041,921, filed April 7, 1997. Prior to calculating the claim fees for the application please enter the following amendments:

In the Claims:

Please amend claims 18, 19, 20, 21, 22 and 26 as follows:

18. (Amended) A use of an immune globulin preparation according to [any one of] claim[s] 1 [to 17] to increase the serum half-life of an immune globulin.

19. (Amended) A use of an immune globulin preparation according to [any one of] claim[s] 1 [to 17] to reduce the elevation of neutrophil counts.

20. (Amended) A method of increasing the serum half-life of an immune globulin comprising administering an immune globulin preparation according to claim[s] 1 [to 17] to an animal in need thereof.

21. (Amended) A method of reducing the elevation of neutrophil counts in a recipient of immune globulin comprising administering an immune globulin preparation according to claim[s] 1 [to 17] to an animal in need thereof.

22. (Amended) A method according to claim 20 [or 21] wherein said immune globulin preparation is administered intravenously.

26. (Amended) The preparation according to [any one of] claim[s] 23[-25] which is aqueous.

Entry of the above preliminary amendment is respectfully requested.

Respectfully submitted,

Hugh W. Price and
B. Michael R. Woloski

M. Gravelle

Micheline Gravelle
Registration No. 40,261

Dated: October 6, 1999

Bereskin & Parr
Box 401, 40 King Street West
Toronto, Ontario, Canada M5H 3Y2

(416) 364-7311

Title: INTRAVENOUS IMMUNE GLOBULIN FORMULATION CONTAINING A NON-IONIC SURFACE ACTIVE AGENT WITH IMPROVED PHARMACOKINETIC PROPERTIES

FIELD OF THE INVENTION

The present invention relates to an improved method and immune globulin formulation containing a non-ionic surface active agent to prolong the serum half-life and to alter the immunomodulatory effect of immune globulin.

BACKGROUND OF THE INVENTION

Immune globulins (also known as immunoglobulins or antibodies) are proteins produced by lymphoreticular tissues. There are 6 known classes of immune globulin: IgG, IgM, IgA, IgD, IgE and secretory IgA. IgG (also known as gamma-globulin) is the most abundant and the most therapeutically relevant class of immune globulin. The primary function of immune globulins is to specifically recognize and bind antigens through reversible bonding thereby facilitating the immune systems ability to eliminate antigens.

IgG is a glycoprotein of approximately 150,000 Daltons consisting of 2 "heavy" (gamma) chains and 2 "light" (kappa or gamma) chains held together by disulphide as well as weak covalent bonds. Within the class of IgG, there are 4 subclasses of IgG1, IgG2, IgG3 and IgG4 comprising about 70%, 15%, 10% and 5% of total IgG in normal human serum respectively. These subclasses possess minor antigenic differences among their "heavy" chains resulting in distinct biological actions.

There are principally two types of immune globulins that are available as therapeutic agents: standard immune serum globulin preparations for general use, and immune globulin preparations that recognize specific antigens for use in specific disorders. Commercial examples of products in the former category include numerous brands of intravenous immune globulin (Gamimune N® by Bayer; Sandoglobulin® by Sandoz; Gammar-IV® by Armour) as well as intramuscular immune globulin (Gamastan® by Cutter; Gammar® by Armour). Examples of

products in the latter category are hepatitis B immune globulin (H-BIG® by Abbott; Hep-B-Gammagee® by MSD; HyperHep® by Cutter), varicella zoster immune globulin (VZIG by Massachusetts Public Health Biologic Labs.), cytomegalovirus immune globulin (CytoGam® by Connaught) and Rh immune globulin (WinRho® and WinRho SD® by Cangene; HypRho-D® by Miles; Gamulin Rh® by Armour; RhoGAM® by Ortho Diagnostics). The primary therapeutic basis for immune globulins is passive immunity conferred to the recipient through the direct introduction of extraneous "ready-made" antibodies. The major clinical utilities of immune globulins are prophylaxis and/or treatment of antigen-associated disorders.

Immune globulins may be prepared by isolation of natural immune globulins from mammalian serum. Immune globulins prepared using Cohn's cold ethanol fractionation method suffers from relatively low product yield and IgG purity. The resultant product contains significant amounts of aggregated immune globulin which combines with complement (also termed anticomplementary activity) and produces adverse reactions in recipients if given by intravenous injection or infusion (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962). Correspondingly, these immune globulin preparations must be injected intramuscularly (therefore termed intramuscular immune globulin). Intramuscular injections are painful. Drug absorption and peak levels of immune globulin (hence onset of therapeutic action) are slow, and approximately half of the injected dose is lost due to local proteolysis and incomplete absorption. Significant amounts of IgA and IgM are also present in Cohn-prepared intramuscular immune globulin preparations which can cause anaphylactic reactions in certain recipients.

Compared with other parenteral routes, such as those just discussed, intravenous injection or infusion of immune globulin is the preferred route of drug administration in the clinical setting due to instant bioavailability and rapid onset of therapeutic protection.

Intravenous immune globulin products differ from intramuscular products in two fundamental ways. First, an intravenous preparation must contain a significantly smaller amount of aggregated immune globulin molecules (over about 94% monomeric immune globulin) thereby causing fewer anticomplementary adverse reactions. Second, the IgG content and product purity of intravenous immune globulin products are significantly higher (over about 95% IgG content) than intramuscular products. The low level of contamination with IgA or IgM in intravenous immune globulin (less than about 40 ug/mL) is also associated with lower incidences of adverse events such as anaphylactic reactions especially in agammaglobulinemic recipients.

Improved methods involving further purification of the Cohn immune globulin fractions (see Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969) were developed to render immune globulin produced from cold ethanol fractionation suitable for intravenous administration. Ultracentrifugation of the immune globulin-containing fraction, or treatment of immune globulin with pepsin, plasmin, a sulfitolytic agent or beta-propriolactone, reduces the anticomplementary activity of the final preparation (see U.S. Patent No. 4,160,763; Barandun, S. et al., *Monogr. Allergy* 9: 39-60, 1975; Stephan, *Vox Sang.* 28: 422-437, 1975; Wells, J.L.V. et al., *Austr. Ann. Med.* 18: 271, 1969; Baumgarten, W. et al., *Vox Sang.* 13: 84, 1967; Merler, E. et al., *Vox Sang.* 13: 102, 1967; Sgouris, J.T. et al., *Vox Sang.* 13: 71, 1967; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962; Nisonoff, A. et al., *Science* 132: 1770-1771, 1960). U.S. Patent No. 3,903,262 describes the reduction of intermolecular disulphide bonds of immune globulin and alkylation of the resultant sulfhydryl groups. Schura of Germany also developed an immune globulin for intravenous injection by adsorption of immune globulin onto hydroxy-ethyl starch. However, these approaches are often technically unfeasible for manufacturing or residual reactants in the final preparation have been shown to cause undesirable outcomes such as a reduction in serum half-life of the immune globulin and elicitation of immunogenic reactions in recipients.

Further improved chromatographic techniques (*e.g.* using DEAE-Sephadex ion-exchange columns in combination with ultrafiltration) were also developed for the manufacture of immune globulins from human plasma to produce immune globulins suitable for intravenous injection or infusion (see Canadian Patent number 1,168,152; Canadian Patent number 1,201,063; Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964). The use of such chromatographic manufacturing processes also substantially increases product yield to over about 90%.

Immune globulin prepared by improved processes may be administered by intravenous injection or infusion as well as other parenteral routes. For instance, Cangene's WinRho® and WinRho SD® are produced using a proprietary anion exchange chromatographic process and are the only commercial anti-Rh_oD immune globulin preparations that can be administered safely by intravenous injection or infusion to humans. This is due to their relatively higher IgG purity and monomeric protein content as well as lower IgA/IgM contamination.

Monoclonal immune globulins can be produced using recombinant and hybridoma techniques (see Canadian Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may also be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody (see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application

PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988).

Immune globulin preparations suitable for parenteral injection commonly consist of an immune globulin distributed in a physiologically compatible medium. This medium may be sterile water for injection (WFI) with or without isotonic amounts of sodium chloride. For example, the recommended diluent for reconstituting commercial intravenous immune globulins such as Iveegam®, Gammagard®, or, Venoglobulin®, is sterile WFI. Sandoglobulin® is supplied with 0.9% (w/v) sodium chloride solution as diluent (see Gahart, B.L. & Nazareno, A.R., Intravenous Medications: a handbook for nurses and allied health professionals, p. 516-521, Mosby, 1997). WinRho SD®, is reconstituted in 0.9% sodium chloride solution for intravenous injection. The immune globulin product by Schura (*supra*) is formulated as a solution of 165 mEq/L sodium ion and 120 mEq/L chloride ion with a final pH of 6.7. The Miles' intravenous immune globulin preparation, Gammimune®, when constituted, has an osmolality of 278 mOsm/L and a pH of 4.0-4.5. U.S. patent Nos. 4,396,608 and 4,499,073 also disclose a low pH (3.5-5.0) and low ionic strength ($\leq$0.001) immune globulin formulation for intravenous injection. The globulin protein concentration in the above preparations ranges from 0.5% to 20%.

Carbohydrates and their derivatives such as glucose, maltose or mannitol may be included in immune globulin formulations to adjust the tonicity of the preparation. For example, maltose (10%) is included in Miles' intravenous immune globulin preparation, Gammimune®, to achieve isotonicity. Sucrose (5%) is included in Sandoz's intravenous immune globulin preparation, Sandoglobulin® and in Armour's intravenous immune globulin preparation, Gammar-IV®. The commercial intravenous immune globulin preparation, Venoglobulin®, contains 50 mg/mL D-sorbitol.

Likewise, amino acids such as glycine or histidine may be added to improve storage stability of the protein. For example, glycine (0.3M) is included in commercial immune globulin preparations such as

American Red Cross' intravenous immune globulin preparations, Polygam® and Polygam S/D®; the intramuscular varicella zoster immune globulin preparation by Massachusetts Public Health Biologic Laboratories; and the intramuscular anti-Rh_oD immune globulin preparations by Armour and Miles. U.S. patents Nos. 4,396,608 and 4,597,966 describe the use of glycine and histidine to stabilize immune globulin formulations. European Patent Application No. EP 392,717 describes the use of mannitol and glycine to stabilize and prevent aggregation of immune globulin in formulation.

The prolongation of storage shelf-life of immune globulin preparations may also be accomplished by the addition of preservatives including organic mercurial derivatives such as thimerosal.

Surface active agents (also termed surfactants or detergents) are compounds that can lower the surface tension of water. All surface active agents are amphipathic possessing a hydrophobic end (*e.g.* one or more hydrocarbon chain(s)) as well as a hydrophilic moiety (which may or may not be ionic). A surface active agent may be classified as anionic, cationic, or non-ionic depending on the nature of its hydrophilic moiety. Soaps with carboxylate or sulphonate groups carry net negative charges and are examples of anionic surface active agents. Benzalkonium, an N-benzyl quaternary ammonium chloride and an antibacterial agent, carries a net positive charge and is an example of a cationic surface active agent. A non-ionic surface active agent contains a neutral group such as a carbohydrate which can hydrogen-bond with water.

Tween® and Span® are two types of non-ionic surface active agent. Agents such as Span® are partial esters of common fatty acids and sugar alcohol anhydrides derived from sorbitol. Agents such as Tween® are derivatives of Span® products with polyoxyethylene chains attached to non-esterified hydroxyl groups. Their hydrophilic property is due to free hydroxyl and/or oxyalkylene groups, and their hydrophobic property is due to their long chain fatty acids. Examples of commonly used Span® type surface active agents are sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60),

sorbitan tristearate (Span 65), sorbitan monooleate (Span 80), and sorbitan trioleate (Span 85). A commonly used member of Tween® type surface active agent, Tween 80, is also known as Polysorbate 80, sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivative, polyoxyethylene sorbitan monooleate, polyethylene oxide sorbitan monooleate, sorethytan monooleate, Sorlate, Monitan or Olothorb. Other examples of polyoxyethylene sorbitan surface active agents comprise polyoxyethylene sorbitan monolaurate (Tween 20 or 21), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60 or 61), polyoxyethylene sorbitan tristearate (Tween 65), and polyoxyethylene sorbitan trioleate (Tween 85).

The safety of non-ionic surface active agents in mammals has been studied extensively and established. Previous acute toxicity studies indicated that the LD50 values for Tween® and Span® type non-ionic surface active agents in rats are relatively high at >15 g/kg for oral ingestion and >1.4 g/kg for parenteral injection (*J. Am. Coll. Toxicol.* 3: 1-82, 1984; Varma, R.K. et al., *Arzneimittelforschung* 35: 804-808, 1985; Farkas, W.R. et al., *Pharmacol. Toxicol.* 68:154-156, 1991). Subacute and chronic toxicity studies also showed minimal toxicity after administration of relatively high oral doses to rats (100-200 mg per kg body weight) (Nityanand, S and Kapoor, N.K., *Indian J. Med. Res.* 69: 664-670, 1979). A number of reproductive toxicology studies also did not identify a hazard with the clinical use of non-ionic surface active agents (Kitchin, K.T. and Ebron, M.D., *Toxicology* 30: 45-47, 1984; Ema, M. et al., *Drug Chem. Toxicol.* 11: 249-260, 1988; Gajdova, M. et al., *Med. Toxicol.* 3: 128-165 and 209-240, 1988).

The inclusion of surface active agents in protein drug preparations has been practised extensively to improve product stability in storage and/or to increase product solubility. A commercially available preparation of granulocyte colony stimulating factor, Neupogen®, contains 0.004% Polysorbate 80 to improve storage stability. Turbersol® is a sterile isotonic solution of Tuberculin in phosphate buffered saline containing 0.0005% Polysorbate 80 as a stabilizer.

With respect to immune globulin preparations, U.S. Patent No. 4,902,500 discloses immune globulin formulations with improved storage stability containing at least one polyoxypropylene-polyoxyethylene block polymer (Pluronic 68). PCT Patent Application FR93/00584 published as WO94/16728 describes the inclusion of Polysorbate 80 in a parenteral formulation of an anti-LFA-1 monoclonal antibody for stabilization purposes. U.S. Patent No. 5,215,743 describes the use of Polysorbate 80 to stabilize parenteral formulations of tumour necrosis factor (TNF).

U.S. Patent No. 5,151,266 teaches a method of treating antibodies with an anionic detergents such as sodium dodecylsulfate (sodium lauryl sulfate), cetyl ammonium sulfate, or taurocholic acid, to increase the solubility of the antibody and to reduce its reticuloendothelial uptake. The method claimed in U.S. Patent No. 5,151,266 involves preincubation of an antibody with anionic detergent: any unreacted anionic detergent is removed before drug administration.

Current commercial anti-Rh_oD immune globulin preparations containing $\leq$0.01% Polysorbate 80 are RhoGAM® and MICRhoGAM® both produced by Ortho Diagnostics Systems Inc. These preparations must be administered only by intramuscular injection due to relatively high protein aggregation and low product purity (as discussed *supra* for such preparations). Similarly, Polygam® and Polygam S/D® (Red Cross) contains $\leq$0.01% Polysorbate 80 to improve immune globulin solubility and storage stability. These products contain relatively low IgG content at 90%.

With respect to the use of surface active agents in the production of immune globulin, U.S. Patent Nos. 4,371,520 and 4,379,086 describes the use of alkylene oxide polymers such as polyethylene glycol in the fractionation process for isolating immune globulin-rich fractions from plasma. Similarly, U.S. Patent Nos. 4,276,283 and 5,132,406 describe the use of alkylene oxide polymers such as polyethylene glycol in a precipitation step for isolating and purifying immune globulin-rich fractions.

The ability of surface active agents to alter the pharmacologic properties of drugs has been examined to a limited extent, but their usefulness in immune globulin formulations in a clinical setting has not been established. Jekunen, A. et al. (*Acta Oncol.* 35: 267-271, 1996) and Kairemo et al. (*Anticancer Res.* 16(6B);3542-3550, 1996) reported the *in vivo* modulation of antibody kinetics in mice by Polysorbate 80. Intra-tumour administration of the non-ionic surface active agent, Polysorbate 80, improved the targeting of a radiolabeled monoclonal antibody to the tumour and accelerated antibody clearance from the blood.

Ellis, A.G. et al. (*Cancer Chemother. Pharmacol.* 38: 81-87, 1996) describes the effects of two surface active agents, Cremophor EL and Tween 80, on the pharmacokinetics of a chemotherapeutic non-protein drug, etoposide, in an isolated perfused rat liver experimental model. Co-administration of either surface active agent decreased the elimination half-life of etoposide. Masters, J.R. et al. (*Cancer Chemother. Pharmacol.* 25: 267-273, 1990) decreased the *in vivo* half-life of the chemotherapeutic drug, thioTEPA in human subjects. These decreases in plasma half-life correspondingly increase the need for more frequent drug administration to maintain effective plasma drug concentrations which in turn increases the cost associated with such therapy in the clinical setting.

Liu, F. and Liu, D. (*Pharm. Res.* 12: 1060-1064, 1995) demonstrated the ability of Polysorbate 80 to attenuate the clearance of parenterally administered oil-in-water emulsions. Such oil-in-water emulsions are physicochemically and biochemically different from the immune globulin proteins of the present invention.

The benefits and methods for covalent bonding of amphipathic polymer moieties to proteins are known in the art. For example, the chemical conjugation of polyethylene glycol (PEG) or monomethoxy-polyethylene glycol (MPEG) to a variety of proteins by a variety of different methods has been described (see PCT Patent Application GB94/01844 published as WO 95/06058; U.S. Patent No. 5,349,052; Delgado, C. et al., *Crit. Rev. Ther. Drug Carrier Sys.* 9: 249-304,

1992). One of the major observed advantages of PEG conjugation to a protein is to decrease its rate of clearance from the body and to increase plasma half-life. However, the polymer (MPEG) by itself without conjugation was shown to elicit no effect on the plasma half-life of proteins.

SUMMARY OF THE INVENTION

Immune globulin preparations with novel clinical characteristics and benefits are presented. Immune globulin preparations of the present invention contain an immune globulin with relatively high IgG and low aggregated protein contents and are suitable for intravenous injection or infusion. The immune globulin preparation contains one or more non-ionic surface active agents in a physiologically compatible buffered medium. Inclusion of one or more such non-ionic surface active agents in the preparation surprisingly prolongs the serum half-life of the immune globulin *in vivo* and improves the safety profile of the product. An immune globulin preparation with an increased serum half-life is clearly advantageous and contrary to the teachings of the prior art. A preparation with an extended half-life means that the active therapeutic ingredient would have a longer survival time in the bloodstream to exert its desired therapeutic effect. A longer serum survival time would also alow for a reduced frequency of drug administration. This results in a more convenient dosing schedule with fewer injections which thereby improves patient compliance. A consequence is to reduce indirect costs associated with parenteral administration of the drug. Moreover, a longer serum survival time of a time may translate into the requirement of smaller maintenance doses to maintain an effective serum drug concentration thereby minimizing the direct cost of drug therapy.

In one aspect, the present invention provides an immune globulin preparation comprising an immune globulin and a non-ionic surface active agent, where the non-ionic surface active agent is in a concentration sufficient to increase the serum half-life of the immune globulin. The immune globulin preparation may have more than one

non-ionic surface active agent, so long as the non-ionic surface active agents are in a concentration sufficient to increase the serum half-life of the immune globulin.

According to one embodiment, the immune globulin is anti-Rh_oD immune globulin wherein the anti-Rh_oD immune globulin has an IgG purity of greater than 95% and a monomeric protein content of greater than 94%. In a preferred embodiment, the preparation is aqueous.

According to another embodiment, the immune globulin is anti-c immune globulin which has an IgG purity of greater than 95% and a monomeric protein content of greater than 94%.

According to another embodiment, the preparation has a concentration of the immune globulin of about 2 weight percent to about 10 weight percent.

The non-ionic surface active agent may be a sorbitan ester of a fatty acid selected from the group consisting of sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan tristearate, sorbitan monooleate and sorbitan trioleate.

The non-ionic surface active agent may also be a polyoxyethylene sorbitan ester of a fatty acid selected from the group consisting of polyoxyethylene (20) sorbitan monolaurate; polyoxyethylene (4) sorbitan monolaurate; polyoxyethylene (20) sorbitan monopalmitate; polyoxyethylene (20) sorbitan monostearate; polyoxyethylene (4) sorbitan monostearate; polyoxyethylene (20) sorbitan tristearate; polyoxyethylene (20) sorbitan monooleate; polyoxyethylene (5) sorbitan monooleate; and polyoxyethylene (20) sorbitan trioleate.

According to one embodiment, the preparation will have a concentration of the non-ionic surface active agent of about 0.01 weight percent to about 0.5 weight percent.

According to another embodiment there is provided an aqueous immune globulin preparation wherein the immune globulin has an increased serum half-life comprising: about 3-8% human anti-Rh_oD immune globulin with an IgG purity of greater than 95% and a monomeric protein content of greater than 94%; sodium chloride at about

0.25% (w/v); very low level buffer with essentially no ionic strength; Polysorbate 80 at about 0.01% to about 0.5% (w/v); and L-glycine at about 0.1M.

In another aspect, the present invention provides a method of increasing the serum half-life of an immune globulin comprising administering a preparation comprising an immune globulin and a non-ionic surface active agent in a physiologically acceptable medium to an animal in need thereof.

In yet another aspect, the present invention provides a method of reducing the elevation of neutrophil counts in a recipient comprising administering a preparation comprising an immune globulin and a non-ionic surface active agent in a physiologically acceptable medium to an animal in need thereof. According to either method the immune globulin preparation may be administered intravenously.

In all cases, the preparation possesses the novel characteristic of an extended serum half-life *in vivo* and reduced immunogenicity in comparison with equivalent immune globulin preparations not containing the non-ionic surface active agent.

A preparation according to the present invention may be in the format of a liquid formulation or may be lyophilized to form a powder formulation. The liquid formulation may be administered directly, while the lyophilized powder format may be reconstituted in a physiologically compatible medium before drug administration.

In an embodiment, the immune globulin in a preparation of the present invention is a human immune globulin prepared by extraction from human plasma using conventional cold ethanol fractionation method followed by a method to render the preparation suitable for intravenous administration or by chromatographic procedures. The immune globulin or binding partner may also be a monoclonal antibody or binding partner produced by recombinant DNA or hybridoma technology.

In another embodiment, the immune globulin in a preparation of the present invention is an anti-D (also known as anti-Rh_o

or anti-Rh_oD) immune globulin; an anti-C (also known as anti-rh') immune globulin; an anti-E (also known as anti-rh") immune globulin; an anti-c (also known as anti-hr') immune globulin or anti-e (also known as anti-hr") immune globulin. This immune globulin may be prepared by conventional cold ethanol fraction followed by a method to render the preparation suitable for intravenous administration, by chromatographic techniques or by recombinant DNA or hybridoma technology.

Another aspect of the invention provides a method of extending the serum half-life or altering the immunomodulatory effect of and immune globulin comprising the addition of a sufficient amount of one or more non-ionic surface active agents to the immune globulin formulation. The immune globulin may a human immune globulin extracted from plasma using conventional ethanol fractionation followed by a method to render the preparation suitable for intravenous administration, by chromatographic methods, or it may be a monoclonal antibody or binding partner produced by recombinant DNA or hybridoma technology. More specifically, the immune globulin may be an anti-D (also known as anti-Rh_o or anti-Rh_oD) immune globulin; an anti-C (also known as anti-rh') immune globulin; an anti-E (also known as anti-rh") immune globulin; an anti-c (also known as anti-hr') immune globulin or anti-e (also known as anti-hr") immune globulin.

In a further aspect of the invention, the immune globulin formulation is administered to a mammal by parenteral injection or infusion to elevate circulating immune globulin levels.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only and are not intended to limit, in any way, the scope of the present invention. As will become apparent to those skilled in the art, various changes and modifications may be made based on the following detailed description, however, all are within the spirit and scope of the prersent invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 is a graph of mean anti-Rh_oD immune globulin blood levels after an intramuscular injection of WinRho SD™. Mean serum anti-Rh_oD immune globulin results are shown for formulations with and without 0.01% (w/v) Polysorbate 80. The solid diamonds show the results from subjects injected with the conventional formulation of WinRho SD™ in 0.9% (w/v) sodium chloride solution. The shaded boxes show the results in subjects injected with new formulation of WinRho SD™ with Polysorbate 80 in 0.9% (w/v) sodium chloride solution.

Figure 2 is a graph of mean anti-Rh_oD immune globulin blood levels for up to 82 days after an intramuscular injection of WinRho SD™. Mean serum anti-Rh_oD immune globulin results are shown for formulations with and without 0.01% (w/v) Polysorbate 80. The solid diamonds show the results from subjects injected with the conventional formulation of WinRho SD™ in 0.9% (w/v) sodium chloride solution. The open squares show the results from subjects injected with the formulation of WinRho SD™ with Polysorbate 80 in 0.9% (w/v) sodium chloride solution.

DETAILED DESCRIPTION OF THE INVENTION

As mentioned above, the present inventors have found that the addition of a non-ionic surface active agent to a preparation of an immune globulin favourably alters the pharmacokinetics of the immune globulin as a therapeutic agent. The inclusion of said non-ionic surface active agent in the formulation prolongs the survival time or serum half-life of said immune globulin.

Accordingly, broadly stated, the present invention provides an immune globulin preparation comprising an immune globulin and a non-ionic surface active agent, where the non-ionic surface active agent is in a concentration sufficient to increase the serum half-life of the immune globulin. The immune globulin preparation may have more

than one non-ionic surface active agent, so long as the non-ionic surface active agents are in a concentration sufficient to increase the serum half-life of the immune globulin.

The phrase "surface active agent" means an agent that reduces surface tension when dissolved in a solution, such as in water or an aqueous solution. The term "surfactant" is synonomous. The phrase "sufficient to increase the serum half-life of the immune globulin" means that the serum half-life of the immune globulin with at least one surface active agent is increased as compared to the serum half-life of the immune globulin when administered without a surface active agent.

The immune globulin of the present invention can be any immune globulin including IgG (all subclasses), IgA, IgD, IgE, and IgM and includes fragments of the immune globulins such as Fab' and $F(ab')_2$ fragments. The immune globulin is preferably non-aggregated (over about 94% monomeric immune globulin) and has a purity of greater than about 95%. More preferably, the immune globulin preparation is in a form suitable for intravenous injection or infusion.

An example of immune globulin that can be used in the present invention is Rh immune globulin or Rh antibodies. Rh antibodies include anti-D (also known as anti-Rh_o or anti-Rh_oD); anti-C (also known as anti-rh'); anti-E (also known as anti-rh''); anti-c (also known as anti-hr') and anti-e (also known as anti-hr''). The Rh antibodies of the present invention may be preparations from plasma enriched for Rh antibodies, polyclonal antibodies, monoclonal antibodies, antibody fragments (e.g. Fab, and $F(ab')_2$), and those produced by recombinant DNA technology. Other immune globulin preparations suitable for intravenous injection or infusion, such as varicella zoster immune globulin (Varitect® by Biotest Pharma) or cytomegalovirus immune globulin (Cytogam® by Connaught), can also benefit from the present invention.

The inventors have also found that the addition of a non-ionic surface active agent to an immune globulin reduced the elevation

of patient neutrophil counts observed with a conventional immune globulin without the non-ionic surface active agent.

Immune Globulin Production

Preparations with a high Rh antibody content suitable for intravenous injection or infusion may be isolated as an immune globulin fraction from plasma, preferably human plasma, using conventional techniques. For example, they may be isolated using: (a) the Cohn cold ethanol fractionation method or modifications thereto (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962); (b) ion-exchange chromatographic methods (*e.g.* using DEAE-Sephadex) and modifications thereto may be used to produce Rh antibodies of higher product yield and quality (Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964); and/or (c) anion-exchange chromatographic method as taught in Canadian Patent No. 1,201,063, and modifications thereto. Commercially available anti-Rh_oD immune globulin preparations may also be used in the methods. For example, anti-Rh_oD preparations such as WinRho® or WinRho SD® (Cangene Corporation) may be used in the present invention.

In an embodiment of the invention, an anti-Rh_oD immune globulin fraction is prepared by contacting an aqueous plasma fraction containing IgG with one or more chromatographic separation columns to produce a purified IgG-rich fraction. The aqueous plasma fraction used in the process may be normal non-immunized plasma from an animal source, preferably a human source, or hyperimmune plasma such as plasma from Rh alloimmunized donors.

For example, Rh_oD antigen is used to immunize an animal through intramuscular, subcutaneous, intraperitoneal, or intraocular injection, with or without an adjuvant such as Freund's complete or incomplete adjuvant. With the option of booster

immunizations, samples of serum are collected and tested for reactivity to the antigen in standard assays (described below). Particularly preferred is polyclonal antisera which will give a signal on one of the assays that is at least three times greater than background. Once the titre of the animal has reached a plateau in terms of antigen reactivity, larger quantities of the antisera may be obtained readily either by periodic bleeding or by exsanguinating the animal.

Human anti-Rh_oD immune globulin may also be produced in human volunteers. For example, an anti-Rh_oD immune globulin preparation may be obtained from a subject who is naturally immunized (*e.g.* from an Rh incompatible pregnancy) or artificially immunized using Rh-positive blood cells or Rh_oD antigen.

Anti-Rh_oD immune globulin-containing plasma collected from animal or human is modified to the ionic strength and pH of the initial buffer used with a chromatographic separation column. According to one embodiment of the invention, an aqueous animal plasma fraction is contacted with one or more, preferably one to two, anionic exchangers to produce a purified IgG-rich fraction.

By way of example, an aqueous animal or human plasma fraction is applied to an anion exchange column which may contain an agarose cross-linked anionic exchange resin such as DEAE-Sepharose CL6B, TMAE Fractogel or DEAE Sephadex A-50. An IgG-rich fraction is obtained from the column by eluting with an equilibrating buffer. The IgG-rich fraction may be concentrated, for example, by ultrafiltration.

The purified IgG protein may optionally be treated with a solvent and detergent to inactivate lipid envelope viruses. Suitable solvents and detergents which may be used include Triton X-100 and tri(n-butyl) phosphate (Horowitz, B., *Curr. Stud. Hematol. Blood Transfus.* 56: 83-96, 1989). After this process, the solvents and detergents may be removed using conventional methods such as reverse phase chromatography.

Monoclonal immune globulins may also be readily produced using recombinant and hybridoma techniques (see Canadian

Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody (see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988). It will be apparent to one skilled in the art that fractionation and recombinant approaches may be applied to diverse types of immune globulins. For example, specific monoclonal immune globulins against different antigens may be generated by techniques based on the same principle of recombinant DNA technology.

Non-Ionic Surface Active Agents

Non-ionic surface active agents of the present invention can be any agent that can prolong the serum half-life of an immune globulin. Preferably, the surface active agent is of the Tween® or Span® type surface active agents.

Span® type agents are partial esters of common fatty acids and sugar alcohol anhydrides derived from sorbitol. The common fatty acids derived from sorbitol are preferably lauric acid, palmitic acid, stearic acid or oleic acid derived from sorbitol. For example, Span 20 is sorbitan monolaurate, Span 40 is sorbitan monopalmitate, Span 60 is sorbitan monostearate, Span 65 is sorbitan tristearate, Span 80 is sorbitan monooleate, and Span 85 is sorbitan trioleate.

Tween® type agents are derivatives of Span® products with polyoxyethylene chains attached to non-esterified hydroxyl groups. The common fatty acids derived from sorbitol are preferably lauric acid, palmitic acid, stearic acid or oleic acid derived from sorbitol. For example,

Tween 20 is polyoxyethylene (20) sorbitan monolaurate, Tween 21 is polyoxyethylene (4) sorbitan monolaurate, Tween 40 is polyoxyethylene (20) sorbitan monopalmitate, Tween 60 is polyoxyethylene (20) sorbitan monostearate, Tween 61 is polyoxyethylene (4) sorbitan monostearate, Tween 65 is polyoxyethylene (20) sorbitan tristearate, Tween 80 is polyoxyethylene (20) sorbitan monooleate, Tween 81 is polyoxyethylene (5) sorbitan monooleate, and Tween 85 is polyoxyethylene (20) sorbitan trioleate.

Non-ionic surface agents such as sorbitan esters or polyoxyethylene sorbitan esters of fatty acids may be prepared by methods well known in the art. Such surface active agents may also be obtained commercially from J.T. Baker Inc. (Phillipsburg, New Jersey, USA), ICI Atkemix (Brantford, Ontario, Canada), Van Waters and Rogers Ltd. (Richmond, British Columbia, Canada), or Nikkol Co. (Japan).

Immune Globulin Preparation

Formulation of anti-Rh_oD immune globulin of the present invention involves the addition of an amount of a non-ionic surface active agent sufficient to extend the serum half-life or to alter the immunomodulatory effect of the immune globulin to the IgG-rich concentrate obtained as described above. The immune globulin preferably is at least about 95% pure, more preferably about 99.5% pure, contains at least 94% monomeric IgG, and has not been subjected to chemical or enzymatic modification. A preferred non-ionic surface active agent is Polysorbate 80 which is added to a final concentration of about 0.01% to about 0.5%. Sodium chloride may be added to the formulation to a final concentration of up to about 0.9%. An additional stabilizing agent such as L-glycine or L-histidine may be added to a final concentration of about 0.025M to 0.05M. A preferred preparation contains the following: a pharmacologically effective amount of human anti-Rh_oD immune globulin (about 3-8%); sodium chloride at about 0.25% (w/v); no or very low level buffer with essentially no ionic strength; Polysorbate 80 at a concentration of about 0.01%-0.02% (w/v); and L-glycine at a concentration of about 0.1M.

The anti-Rh_oD immune globulin formulation is aseptically filtered again through a 0.22 micron filter and put into vials or ampoules. Filling operations are conducted under aseptic conditions and the fill volume per vial is calculated so that each vial contains a pharmacologically effective amount of anti-Rh_oD immune globulin. This specific amount or volume can vary depending upon the intended route of administration and therapeutic use. The target filling volume is also calculated with sufficient excess to allow for variation in the potency assay and/or possible loss of potency during storage.

The final aqueous formulation may be lyophilized using a Virtus 251 SRC-5 Sublimator (or equivalent). Lyophilization (also termed vacuum freeze-drying or sublimation) is commonly used in the manufacture of protein pharmaceuticals to improve the stability of the product and extend its shelf life. The lyophilization process is often described as being divided into three stages: freezing; primary drying (also termed ice sublimation); and secondary drying (also termed water desorption). The starting aqueous solution containing the protein is frozen and the ice is subsequently sublimed thereby leaving a dry porous mass of protein which is stable and can be reconstituted rapidly in water. The technical parameters of the lyophilization process including temperature (eutectic and collapse), vacuum pressure and atmospheric gas composition, are fully automated by the Virtus 251 SRC-5 Sublimator. The basic theories and more practical aspects of protein lyophilization and formulation are described in detail in Skrabanja, A.T. et al. (*J. Pharm. Sci. Technol.* 48: 311-317, 1994); Rey, L.R. (*Dev. Biol. Stand.* 74: 3-8, 1992); Pikal, M.J. (*BioPharm* October, 26-30, 1990); Pikal, M.J. (*J. Parenter. Sci. Technol.* 39: 115-138, 1985); Williams, N.A. and Polli, G.P. (*J. Parenter. Sci. Technol.* 38: 48-59, 1984; Nail, S.L. (*J. Parenter. Drug Assoc.* 34: 358-368, 1980); Ito, K. (*Chem. Pharm. Bull.* 19: 1095-1102, 1971).

In the case of a lyophilized powder formulation, the powder comprising the immune globulin and the non-ionic surface active agent is reconstituted in a physiologically compatible diluent such as sterile water for injection or saline before parenteral administration.

For example, 120 ug (600 IU) or 300 ug (1,500 IU) of a commercial anti-Rh_oD immune globulin product, WinRho SD™, is reconstituted in 2.5 mL diluent.

Therapeutic dosage of immune globulin preparation

Dosages of anti-Rh_oD immune globulin in formulations of the present invention depend on individual needs, on the protein content/concentration of the immune globulin preparation, on the desired effect in a particular therapeutic indication, and on the chosen route of drug administration. Daily dosages of an anti-Rh_oD immune globulin preparation (3% to 8% wt-solution) for humans by intramuscular or intravenous injection generally vary between about 50 IU (10 ug) to 2,000 IU (400 ug) per kg body weight. For intramuscular injection, the preferred dosage is about 100 IU (20 ug) to 2,000 IU (400 ug) per kg body weight. For intravenous injection, the preferred dosage is about 50 IU (10 ug) to 1,000 IU (200 ug) per kg body weight, preferably 250 IU (50 ug) per kg body weight. The recommended dosage of Biotest Pharma's intravenous varicella zoster immune globulin preparation, Varitect®, is about 50 IU per kg body weight for shingles therapy and are lower (about 12 to 25 IU per kg body weight) for chickenpox prophylaxis. In contrast, the recommended dosages of general intravenous immune globulin products (4.5-5.5 wt-% solution) such as Gamimune®, Iveegam® or Sandoglobulin® are significantly higher at about 100 mg to 800 mg per kg body weight.

EXAMPLES

EXAMPLE 1

Pharmacokinetics of anti-Rh_oD immune globulin given as Polysorbate 80 formulation over a period of 30 days

Methods. The Pharmacokinetics of anti-Rh_oD immune globulin formulations with and without Polysorbate 80 were assessed in a single-centre, randomized, parallel arm study. Twenty-four human subjects (normal, healthy male and female volunteers of age 18 to 55 years) were randomized into two groups to receive 600 µg (3,000 IU) of a commercial brand of anti-Rh_oD immune globulin, WinRho SD®. Twelve subjects

received the conventional WinRho SD® formulation without Polysorbate 80, and the other 12 subjects received the WinRho SD® formulation with 0.01% (w/v) Polysorbate 80. Each formulation was given intramuscularly as two 1.25 mL injections with the test articles being lyophilized human anti-Rh_oD immune globulin in 0.9% (w/v) saline for intravenous injection with or without Polysorbate 80.

Screening assessments were conducted within three weeks of drug administration. Baseline assessments were conducted on the morning of the day that WinRho SD™ was administered to the subjects and before drug administration. These included haematology, blood chemistry and urinalysis. Demographics, vital signs and baseline laboratory tests were compared for study subjects randomized to the different arms of treatment. There was no statistically significant difference between the two treatment groups in any assessments prior to drug administration.

Subjects remained under observation for eight hours and blood samples for anti-Rh_oD immune globulin analysis were drawn from the study subjects to provide 5 mL serum samples at the following times after study drug administration: 8 hours, 24 hours, 3 days, 7 days, 11 days, 14 days, 21 days, and 28 days. Subjects also underwent haematology, blood chemistry and urinalysis laboratory testing at 7 days and 28 days after WinRho SD™ injection. Anti-Rh_oD immune globulin concentration in patient samples was analyzed by conventional techniques (see Auto-Analyzer technique in Moore, B.P.L., *Can. Med. Assoc. J.* 100: 381-387, 1969).

Pharmacokinetics. Regressions were performed on log transformed corrected serum anti-Rh_oD immune globulin levels against time to determine lambda and subsequently, the estimated half-life of drug in the study subjects. Blood levels after the day 3 draw were used in these regressions as peak serum levels of anti-Rh_oD immune globulin were usually obtained by day 3 after injection of drug. Significant linear relationships ($p<0.02$) existed between the variables Log (corrected anti-Rh_oD immune globulin) and Time for all study subjects.

The mean time to peak anti-Rh_oD immune globulin levels after intramuscular injection of 600 μg of WinRho SD™ was about 3 days and peak serum anti-Rh_oD immune globulin levels that were achieved were about 70 ng/mL. There was no statistically significant difference between the two formulations and the mean time to peak or peak levels of drug. Similarly, the $AUC_{28\ day}$ was about 1250 days•ng/mL and there was no difference between the two formulations in these values.

The mean half-life was different for the two formulations of WinRho SD™. The formulation of drug without Polysorbate 80 had a mean apparent serum half-life of 16.4 ± 3.8 days, while the mean apparent half-life in subjects whom received the formulation with Polysorbate 80 was 20.3 ± 3.4 days. This difference is statistically significant (p=0.012) in a Student t test of the difference between means. Key pharmacokinetic data are presented in Table 1.

TABLE 1

	Formulation without Polysorbate 80	Formulation with Polysorbate 80	Significance
Time to Peak (days) Mean ± SD Median Range	 2.83 ± 0.14 3.00 1.00 - 3.04	 3.65 ± 1.55 2.99 2.96 - 6.99	p=0.100
Peak Anti-D (ng/mL) Mean ± SD Median Range	 76.2 ± 12.2 79.7 54.76 - 99.09	 68.8 ± 11.8 68.8 49.28 - 88.39	p=0.157
AUC (days•ng/mL) Mean ± SD Median Range	 1225 ± 195 1278 938 - 1564	 1273 ± 268 1174 982 - 1803	p=0.673
Serum Half-Life (days) Mean ± SD Median Range	 16.4 ± 3.6 17.0 9.04 - 21.91	 20.3 ± 3.4 20.6 15.07 - 26.74	P=0.012

Pharmacokinetics of anti-Rh_oD immune globulin given as Polysorbate 80 formulation over a period of 84 days.

5 mL serum samples were also collected from the same subjects on 42, 56 and 84 days post injection and serum anti-Rh_oD immune globulin levels were determined for up to 84 days post administration of WinRho SD™.

Individual subject pharmacokinetics are presented in Table 2. The slope from the regression of log (corrected anti-D) on actual time after injection of WinRho SD (up to day 84) data was included in the regression analysis.

TABLE 2

Subject	Time to Peak (Days)	Peak Anti-D (ng/mL)	Corrected Peak Anti-D (ng/mL)	λ (/day)	$t_{1/2}$ (days)	AUC (days•ng/mL)
WinRho SD™ Formulation (without Polysorbate 80)						
SS02	3	91.54	83.22	0.040118	17.278	1940.45
SS03	3	80.04	72.76	0.040598	17.073	1853.01
SS05	1	88.67	79.06	0.028732	24.125	2544.84
SS06	2.99	89.3	81.18	0.050862	13.628	1135.8
SS08	2.98	62	56.36	0.033751	20.537	1597.15
SS10	3	55.42	54.76	0.032355	21.423	1435.6
SS14	2.98	86.74	78.85	0.035784	19.37	1979.34
SS16	2.96	66.71	70.19	0.035764	19.381	1489.86
SS18	2.99	95.05	99.09	0.036003	19.252	2263.9
SS19	3.03	85.31	84.67	0.029916	23.17	2034.25
SS20	3.04	80.59	81.04	0.040478	17.124	1866.07
SS22	3	74.15	71.41	0.042734	16.22	1600.78
WinRho SD™ Formulation (with Polysorbate 80)						
SS01	3	82.8	75	0.031157	22.247	2131.76
SS04	3.08	66.56	60.29	0.027221	25.464	1769.77
SS07	6.97	88.32	80	0.018439	37.591	3043.64
SS09	2.96	77.23	69.95	0.031561	21.962	1759.42
SS11	2.96	82	74.28	0.033716	20.558	1986.99
SS12	6.99	97.58	88.39	0.02761	25.105	2963.95
SS13	3	91.59	82.96	0.035027	19.789	2300.9
SS15	2.96	74.57	67.55	0.028825	24.047	1998.26
SS17	2.96	62.85	56.93	0.033925	20.432	1702.44
SS21	2.98	71.01	64.32	0.033193	20.882	1710.56
SS23	2.99	62.97	57.04	0.030317	22.863	1679.9
SS24	2.98	54.41	49.28	0.024491	28.302	1737.46

Mean peak values of anti-Rh_oD immune globulin levels were 79.6 ± 12.6 ng/mL for the conventional formulation and 76.0 ± 13.0 ng/mL for the new formulation which are not significantly different (t = -0.69603; p = 0.4937). Mean time-to-peak values were 3.65 ± 2.56 days for the conventional formulation and 2.83 ± 0.58 days for the new formulation which are not significantly different (t = -1.71648; p = 0.10012). Similarly, mean area under the curve ($AUC_{84\ day}$) values were 1811.8 ± 385.3 days for the conventional formulation and 2065.4 ± 479.6 days.ng/mL for the new formulation which are not significantly different (t = -1.4284; p = 0.16722). Conversely, the mean apparent half-life values for the two WinRho SD™ formulations were different. The formulation of drug without Polysorbate 80 had a mean apparent serum half life of 19.1 ± 3.00 days, while the corresponding value for the drug formulation with Polysorbate 80 was 24.1 ± 4.9 days (t = -3.03582; p = 0.006).

Safety Of Administration

Twenty-four subjects completed 28 days of participation in this study and no subject was withdrawn because of adverse experience. There were a total of 33 adverse events reported in this study (see Table 3). The majority of the events occurred in the Body As A Whole (13 events) and the Respiratory System (9 events). For the most part, the events were evenly distributed between the two different arms of treatment. An exception was 3 adverse events that occurred in the Digestive System: two reports of dyspepsia and one report of vomiting occurred in subjects receiving WinRho SD™ without polysorbate 80. However, since none of the events that occurred in the Digestive System were believed to be related to the WinRho SD™ injection, this observation is not considered significant.

TABLE 3

Preferred Term	All Reports		Formulation without Polysorbate 80		Formulation with Polysorbate 80	
	# Events	# (%)	# Events	# (%)	# Events	# (%)
BODY AS A WHOLE	13	13 (54%)	6	6 (50%)	7	7 (58%)
CARDIOVASCULAR SYSTEM	1	1 (4%)	0	0	1	1 (8%)
DIGESTIVE SYSTEM	3	3 (12%)	3	3 (25%)	0	0
NERVOUS SYSTEM	3	3 (12%)	2	2 (17%)	1	1 (8%)
RESPIRATORY SYSTEM	9	6 (25%)	4	3 (25%)	5	3 (25%)
SPECIAL SENSES	2	2 (8%)	0	0	2	1 (17%)
UROGENITAL SYSTEM	2	1 (4%)	2	1 (8%)	0	0

On the morning of study drug administration and before drug injection, a baseline assessment was conducted which included Vital Signs. The Vital Signs were then assessed in study subjects at 1 hour, 3 hour, 8 hour, 24 hour, 7 day and 28 days after drug administration. There were no statistically significant changes in group Vital Signs from baseline and all mean group changes in Vital Signs were within a standard deviation of zero. The largest changes in vital signs relative to the variance was in the temperature of the subjects receiving WinRho SD™ without polysorbate 80 at early times after drug administration. Body temperature was elevated by 0.3±0.4 °C at 1 hour, 0.3±0.3 °C at 3 hours and 0.5±0.5 °C at 8 hours after WinRho SD™ injection compared to increases in this group of 0.2±0.4 °C at 24 hours, 0.1±0.6 °C at 7 days and 0.1±0.4 °C at 28 days after drug administration. In contrast, the study subjects who received WinRho SD™ with polysorbate 80 had body temperature increases of 0.0±0.5 °C, 0.1±0.5 °C, 0.2±0.5 °C, 0.0±0.4 °C, 0.1±0.7 °C and 0.1±0.4 °C at the assessments after drug administration. Given the low statistical significance to these changes in body temperature, it is not clear if they are related to WinRho SD™ administration. If they are, then the changes are subclinical and the pyrogenic effect is smaller with the new formulation of WinRho SD™, i.e., WinRho SD™ with polysorbate 80.

On the morning of study drug administration and before drug injection, a baseline assessment was conducted that included

laboratory testing. These laboratory tests were repeated in study subjects at 7 days and 28 days after injection of WinRho SD™. Day 7 laboratory data was not significantly different from baseline data and mean Δ parameter values were within a standard deviation of zero. However, the mean neutrophil count for the patients receiving the conventional, or old formulation, i.e., WinRho SD™ without polysorbate 80, was 4.67% ± 4.72% seven days after drug administration and this change was statistically different (p=0.040) from the mean increase of -0.75% ± 7.20% in neutrophils 7 days after administration of the new formulation of WinRho SD™. This difference accounted, in part, for the differences between arms in the proportion of WBC that were neutrophils (61% *vs.* 53%) and in the bands ($4.44x10^9$/L *vs.* $3.07x10^9$/L) at 7 days after drug administration.

There are no statistically significant differences in treatment arms in the haematology laboratory data obtained 28 days after drug administration. There was an overall decrease of 1.3% in the haematocrit of the study subjects 28 days after WinRho SD™ that may have been related to the frequent phlebotomy associated with participation in the study.

There were differences in the 7 day ΔALT and ΔBilirubin with data from the subjects receiving new formulation being closer to baseline values than data from subjects receiving old formulation. However, the mean differences reflected subclinical changes and there were no differences in mean ALT and mean Bilirubin data for these groups. As such, these differences were believed to be fortuitous and result from the frequent hypothesis testing in this analysis. There were no statistically significant differences between the treatment arms in the clinical chemistry data obtained 28 days after study drug administration.

Conclusion. The formulation of WinRho SD™ that contains Polysorbate 80 had the same, or a better, safety profile as the currently licensed WinRho SD™. This is consistent with the relatively high LD50 values for non-ionic surface active agents in mammals (*supra.*). This new formulation also improved the appearance and stability of WinRho SD™

when reconstituted. Surprisingly, the formulation with Polysorbate 80 has a longer half-life (20.3 days) than the formulation without Polysorbate 80 (16.4 days). This difference is beneficial in the therapeutic use of the drug. For example, the new WinRho SD™ formulation with Polysorbate 80 leads to higher passive anti-Rh_oD immune globulin levels for long times after drug administration in prophylaxis of Rh Immunization of Rh negative patients. Moreover, inclusion of Polysorbate 80 in the anti-Rh_oD immune globulin preparation significantly minimized drug-induced elevations of neutrophil counts in the recipients and altered the immunomodulatory effect of the immune globulin.

While the present invention has been described with reference to what are presently considered to be preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

AMENDED CLAIMS

[received by the International Bureau on 8 February 1999 (08.02.99);
original claims 17-21 replaced by amended claims 18-22;
remaining claims unchanged (4 pages)]

1. An immune globulin preparation comprising an immune globulin and at least one non-ionic surface active agent, said one or more non-ionic surface active agent(s) in a concentration sufficient to increase the serum half-life of the immune globulin.

2. The preparation according to claim 1 wherein the immune globulin is anti-Rh_oD immune globulin.

3. The preparation according to claim 2 wherein the anti-Rh_oD immune globulin has an IgG purity of greater than about 95% and a monomeric protein content of greater than about 94%.

4. The preparation according to claim 3 which is aqueous.

5. The preparation according to claim 1 wherein the immune globulin is anti-c immune globulin.

6. The preparation according to claim 5 wherein the anti-c immune globulin has an IgG purity of greater than about 95% and a monomeric protein content of greater than about 94%.

7. The preparation according to claim 6 which is aqueous.

8. The preparation according to claim 1 wherein the concentration of the immune globulin is about 2 weight percent to about 10 weight percent.

9. The preparation according to claim 1 wherein the one or more non-ionic surface active agent(s) is(are) a sorbitan ester of a fatty acid.

10. The preparation according to claim 9 wherein the non-ionic surface active agent(s) is(are) selected from the group consisting of sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan tristearate, sorbitan monooleate, and sorbitan trioleate.

11. The preparation according to claim 1 wherein the one or more non-ionic surface active agent(s) is(are) a polyoxyethylene sorbitan ester of a fatty acid.

12. The preparation according to claim 11 wherein the non-ionic surface active agent(s) is(are) selected from the group consisting of polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (4) sorbitan monolaurate, polyoxyethylene (20) sorbitan monopalmitate, polyoxyethylene (20) sorbitan monostearate, polyoxyethylene (4) sorbitan monostearate, polyoxyethylene (20) sorbitan tristearate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene (5) sorbitan monooleate, and polyoxyethylene (20) sorbitan trioleate.

13. The preparation according to claim 1 wherein two or more non-ionic surface active agents are selected from the group consisting of polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (4) sorbitan monolaurate, polyoxyethylene (20) sorbitan monopalmitate; polyoxyethylene (20) sorbitan monostearate, polyoxyethylene (4) sorbitan monostearate, polyoxyethylene (20) sorbitan tristearate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene (5) sorbitan monooleate, and polyoxyethylene (20) sorbitan trioleate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan tristearate, sorbitan monooleate, and sorbitan trioleate.

14. The preparation according to claim 1 wherein the concentration of the one or more non-ionic surface active agent(s) is(are) about 0.01 weight percent to about 0.5 weight percent.

15. The preparation according to claim 1 wherein the aqueous immune globulin preparation is lyophilized to form a dry powder preparation.

16. An aqueous immune globulin preparation wherein the immune globulin has an increased serum half-life comprising:

about 3-8% human anti-Rh_oD immune globulin with an IgG purity of greater than 95% and a monomeric protein content of greater than 94%;

sodium chloride at about 0.25% (w/v);

very low level buffer with essentially no ionic strength;

Polysorbate 80 at about 0.01% to about 0.5% (w/v); and

L-glycine at about 0.1M.

17. The preparation according to claim 1 wherein the one or more non-ionic surface agents are selected from the group consisting of glyceryl monooleate; and a polyvinyl alcohol.

18. A use of an immune globulin preparation according to any one of claims 1 to 17 to increase the serum half-life of an immune globulin.

19. A use of an immune globulin preparation according to any one of claims 1 to 17 to reduce the elevation of neutrophil counts.

20. A method of increasing the serum half-life of an immune globulin comprising administering an immune globulin preparation according to claims 1 to 17 to an animal in need thereof.

21. A method of reducing the elevation of neutrophil counts in a recipient of immune globulin comprising administering an immune globulin preparation according to claims 1 to 17 to an animal in need thereof.

22. A method according to claim 20 or 21 wherein said immune globulin preparation is administered intravenously.

1/2

FIGURE 1

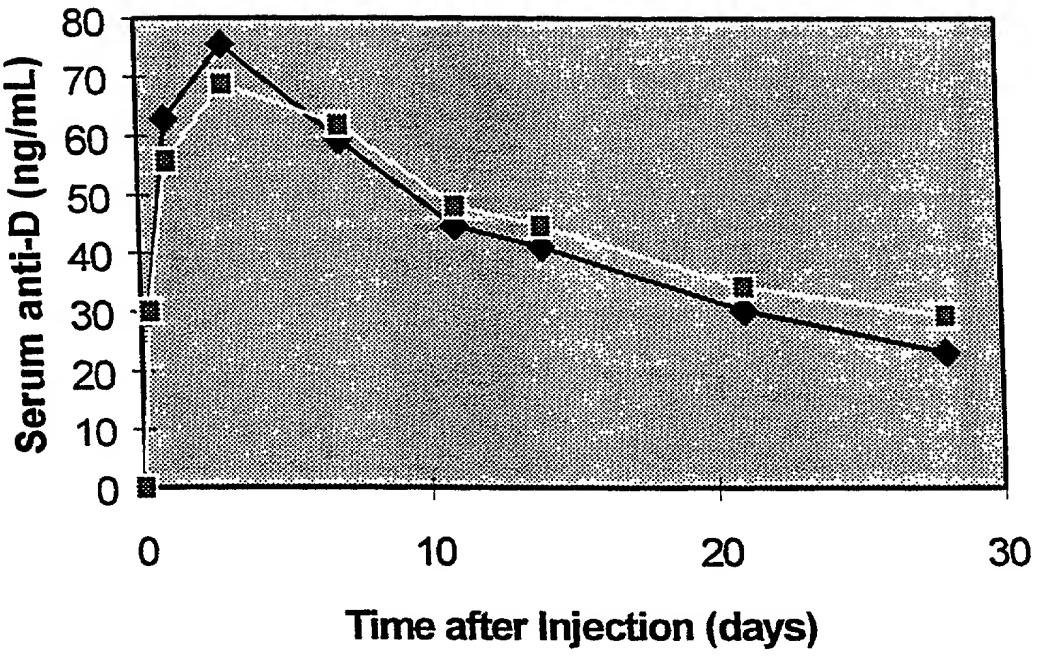
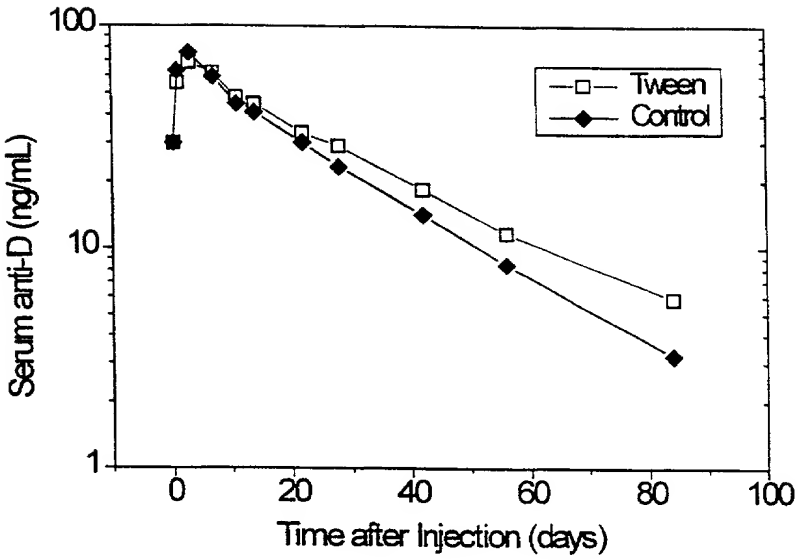

Serum anti-D (ng/mL)
80
70
60
50
40
30
20
10
0
0
10
20
30
Time after Injection (days)

FIGURE 2


100
10
1
Serum anti-D (ng/mL)
0
20
40
60
80
100
Time after Injection (days)
Tween
Control

BERESKIN & PARR BOX 401, 40 KING STREET WEST, TORONTO, CANADA M5H 3Y2
PHONE (416) 364-7311 • FAX (416) 361-1398 • WWW.BERESKINPARR.COM

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☑ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	7841-89/MG
First Named Inventor	Hugh W. Price
COMPLETE IF KNOWN	
Application Number	09/402,446
Filing Date	October 7, 1999
Group Art Unit	N/A
Examiner Name	N/A

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Intravenous Immune Globulin Formulation Containing a Non-Ionic Surface Active Agent With Improved Pharmacokinetic Properties

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☑ was filed on (MM/DD/YYYY) 10/07/99 as United States Application Number or PCT International Application Number 09/402,446 and was amended on (MM/DD/YYYY) _____ *(if applicable)*.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)
60/041,921	04/07/97

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/CA98/00325	04/07/98	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☑ Customer Number 1059 → *Place Customer Number Bar Code Label here*
OR
☐ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Daniel R. Bereskin	22,257	H. Samuel Frost	31,696
Richard J. Parr	22,836	Philip Mendes da Costa	33,106
H. Roger Hart	26,426	Robert B. Storey	33,108
David W.R. Langton	27,747	John R. Rudolph	38,003
C. Lloyd Sarginson	29,245	Robin L.A. Coster	38,016
Timothy J. Sinnott	31,083	Michael E. Charles	38,036

☑ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☑ Customer Number or Bar Code Label 1059 *OR* ☐ Correspondence address below

Name	Bereskin & Parr				
Address	Box 401				
Address	40 King Street West				
City	Toronto	State	Ontario	ZIP	M5H 3Y2
Country	Canada	Telephone	(416) 364-7311	Fax	(416) 361-1398

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Hugh W.	Price

Inventor's Signature	X					Date	00/01/11
Residence: City	Winnipeg	State	Manitoba	Country	Canada	Citizenship	Canadian
Post Office Address	350 Kingston Crescent						
Post Office Address							
City	Winnipeg	State	Manitoba	ZIP	R2M 0T8	Country	Canada

☑ Additional inventors are being named on the ___ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Barry Michael Ronald			Woloski					
Inventor's Signature							**Date**	09/01/10
Residence: City	Winnipeg	**State**	Manitoba	**Country**	Canada		**Citizenship**	Canadian
Post Office Address	8 Everden Road							
Post Office Address								
City	Winnipeg	**State**	Manitoba	**ZIP**	R2N 3Y2	**Country**	Canada	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Inventor's Signature							**Date**	
Residence: City		**State**		**Country**			**Citizenship**	
Post Office Address								
Post Office Address								
City		**State**		**ZIP**		**Country**		

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Inventor's Signature							**Date**	
Residence: City		**State**		**Country**			**Citizenship**	
Post Office Address								
Post Office Address								
City		**State**		**ZIP**		**Country**		

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Please type a plus sign (+) inside this box → +

PTO/SB/02C (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION	REGISTERED PRACTITIONER INFORMATION (Supplemental Sheet)

Name	Registration Number	Name	Registration Number
Micheline Gravelle	40,261	Robert H.C. MacFarlane	40,366
Andrew I. McIntosh	40,453	Stephen M. Beney	41,563
Shawn D. Jacka	43,379		

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.